GRISEOLIC ACIDS B AND C, AN INHIBITOR OF CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE PHOSPHODIESTERASE

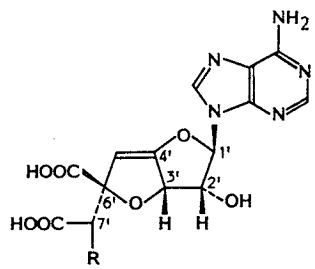
Sir:

In the previous paper, we reported the structure **1** of griseolic acid A (previously reported as griseolic acid[1]) produced by *Streptomyces griseoaurantiacus* SANK 63479. In this paper, we report the isolation procedure and structural elucidation of griseolic acids B[2] and C[3].

Griseolic acids B and C were separated from the mother liquid of griseolic acid A by Sephadex G-10 column chromatography. The mother liquid was dissolved in water and applied on Sephadex G-10 column and developed with water. Two active fractions (griseolic acids B and C) were individually chromatographed on a column of DEAE-Sephadex A-25. After washing with water, the column was eluted with 0.3 M NaCl. The active eluate was then adsorbed on a Diaion HP-20 column. The column was washed with water and eluted with 60% Me_2CO. The eluate was concentrated *in vacuo* and then chromatographed on a Sephadex LH-20 column with water. The active eluate was concentrated *in vacuo* and stored at 4°C overnight to give colorless crystals.

Griseolic acid B (**2**): $C_{14}H_{13}N_5O_7$; mp 160°C (dec); $[\alpha]_D^{20}$ +13.2° (*c* 1.1, DMSO); fast atom bombardment mass spectra (FAB-MS) *m/z* 364 $(M+H)^+$, and griseolic acid C (**3**): $C_{14}H_{15}N_5O_7$; mp 160°C (dec); $[\alpha]_D^{20}$ −50.7° (*c* 1.0, DMSO); FAB-MS *m/z* 366 $(M+H)^+$ were both crystalline. Griseolic acids B (**2**) and C (**3**) were both attributed to have a N-9 substituted adenine moiety in view of the UV absorption at max 256 nm in 0.01 N HCl and 260 nm in 0.01 N NaOH, ^1H NMR and ^{13}C NMR spectra, which included signals at 8.21 and 8.33 ppm for ^1H NMR spectra **2** and **3**, and 155.8 (s, C-6), 152.8 (d, C-2), 148.4 (s, C-4), 139.5 (d, C-8) and 118.6 (s, C-5) in **2** and 155.7 (s, C-6), 152.5 (d, C-2), 148.5 (s, C-4), 139.6 (d, C-8) and 118.7 (s, C-5) in **3** for ^{13}C NMR spectra. In addition, the FAB-MS of **2** and **3** showed a fragment peak at *m/z* 136 corresponding to an adenine moiety. ^1H NMR spectra (Table 1) of griseolic acids A (**1**), B (**2**) and C (**3**) show close similarities.

On comparison of the ^1H NMR spectra of **2** with that of **1**, it was noted that the singlet at 4.7 ppm in **1** (representing a C-7′ proton) was not present in **2**, and the doublet doublets of AB type (2.78 and 3.08 ppm) appeared instead. This observation suggested that the structure of griseolic acid B (**2**) corresponded to 7′-deoxygriseolic acid A. In the ^{13}C NMR spectrum of griseolic acid C (**3**), sp^2 carbons at 97.3 and 157.7 ppm of **2** disappeared and sp^3 carbons at 85.6 (or 77.9) and 35.7 ppm newly appeared as a doublet and triplet. A C-5′ olefinic proton at 5.08 ppm in the ^1H NMR spectrum of **2** was absent in **3**; this indicated the presence of a methylene group (2.3 ppm) and a methine bearing oxygen (4.46 ppm) in **3**. These spectral properties suggested that the structure of griseolic acid C (**3**) corresponded to dihydrogriseolic acid B. The structure of griseolic acid C (**3**) was deduced by X-ray analysis. Crystal date are as follows: $C_{14}H_{15}N_5O_7 \cdot 2H_2O$, MW 401.4, monoclinic, $P2_1$, a=13.660(2), b=8.509(1), c=7.881(2) Å, β=106.6(1)°, U=887.9 Å^3, Z=2, D_{calc}=1.52 gcm^{-3}, μ(CuKα)=1.1 mm^{-1}. The structure was solved by MULTAN[4] and refined



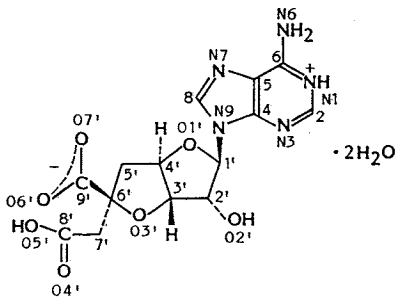
Griseolic acid A (**1**) R = OH
Griseolic acid B (**2**) R = H

Table 1. ^1H NMR data of griseolic acids.

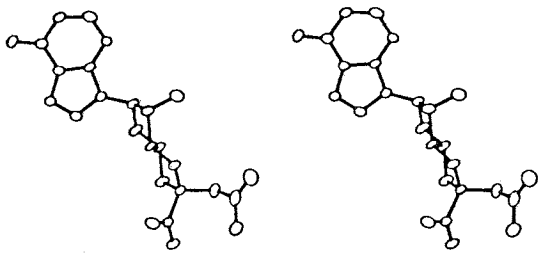
	C-1′	C-2′	C-3′	C-5′	C-7′	C-4′
Griseolic acid A (**1**)	6.67 (s)	4.77 (d)	5.79 (q)	5.31 (d)	4.70 (s)	
Griseolic acid B (**2**)	6.49 (s)	4.60 (d)	6.0 (q)	5.08 (d)	2.78, 3.08 (AB, q)	
Griseolic acid C (**3**)	6.20 (s)	4.46 (ov)	4.46 (ov)	2.30 (AB, q)	2.94 (AB, q)	4.46 (ov)

s: Singlet, d: doublet, q: quartet, ov: overlap.

Fig. 1. Structure of griseolic acid C.



Griseolic acid C (**3**)



by block-diagonal least-squares method (the final R facter: 0.068). Fig. 1 shows the conformation of **3**. It is a zwitterion with the base site N1 protonated by a carboxylate hydrogen attached to β-position of C-6'. The bond lengths and angles in the adenine ring are similar to the values found a typical N1 protonated adenine derivative[5]. The adenine base is *anti* with respect to the sugar moiety, with glycosidic torsion angle χ_{CN} 38.4°. And the two five membered rings of the sugar moiety are trans-fused to each other with torsion angle H-3' – C-3' – C-4' – H-4' of 179.7°. There was a negative cotton effect in the CD spectra of both **1** and **3**, indicating that they both had the same absolute configuration.

Inhibitory activities of griseolic acids A, B, C and papaverine against cyclic adenosine 3',5'-monophosphate phosphodiesterase from rat brain [EC 3.1.4.17] expressed in terms of 50% inhibition (IC_{50}, μM) were 0.16, 0.16, 0.12 and 3.5, respectively.

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